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Sex-specific Effects of Unpredictable Variable Prenatal Stress: Implications for Mammalian Developmental Programming During Spaceflight

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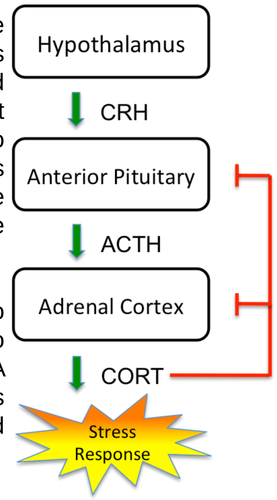
INTRODUCTION

During adaptation to the microgravity environment, adult mammals experience stress mediated by the Hypothalamic-Pituitary-Adrenal axis. In our previous studies of pregnant rats exposed to 2-g hypergravity via centrifugation, we reported decreased corticosterone and increased body mass and leptin in adult male, but not female, offspring. In this study, we utilized Unpredictable Variable Prenatal Stress to simulate the stressors of spaceflight by exposing dams to different stressors. Stress response modulation occurs via both positive and negative feedback in the hypothalamus, anterior pituitary gland, and adrenal cortex resulting in the differential release of corticosterone (CORT), a murine analog to human cortisol.

In this study, we exposed non-manipulated, Gestational Day 0 (G0) dams to Unpredictable Variable Prenatal Stress (UVPS), raising the resultant offspring to Postnatal Day 90 (P90) followed by sacrifice and processing of tissues for RNA purification, cDNA synthesis, and RT-qPCR. In addition to the primary HPA axis genes resulting in CORT release, the following genes were additionally analyzed via RT-qPCR in the same tissues:



Organ	Gene of Interest	Target	Effect
Hypothalamus	Corticotropin-Releasing Hormone	Anterior Pituitary	Primary stress response regulator.
	Arginine Vasopressin	Kidneys / Vessels	Anti-diuretic hormone; peripheral vasoconstriction
	Glucocorticoid Receptor	CORT	Down-regulates inflammatory protein gene expression
	Pro-opiomelanocortin	Systemic	Cleaved into multiple proteins involved in homeostasis and immunomodulation
	Brain-Derived Neurotrophic Factor	CNS / PNS	Modulation of neuron development and long-term memory
Pituitary	CRH Receptor	CORT	Primary receptor for CORT
	Vasopressin Receptor 1B	AVP	Primary receptor for vasopressin, stimulates ACTH release.
Adrenal Glands	Tyrosine Hydroxylase	L-tyrosine	Catalyzes L-tyrosine into L-DOPA, involved in dopamine and catecholamine synthesis.

METHODS

1. Prenatal Stress
2. Maturation to P90
3. Sacrifice and Tissue Processing
4. RNA Purification and cDNA Synthesis
5. RT-qPCR

1. Dams were exposed to three different stressors: (1) White Noise, (2) Strobe Light, and (3) Tube Restraint. Stressors were applied from Gestational Day 0 (G0), following an unpredictable schedule (morning [0600-1200hrs]; afternoon [1200-1800hrs]; evening [1800-2400hrs] in 15, 30, or 60 minute durations alongside non-stressed (NS) control dams.
2. Pups were fostered to newly-parturient, non-manipulated dams to control for differential maternal care.
3. On Post-Gestational Day 90 (P90), offspring were sacrificed and relevant tissues harvested for gene analysis.
4. RNA was extracted from tissues using the Qiagen RNeasy Mini Kit. cDNA was synthesized from purified RNA and normalized to 50. RT-qPCR was performed on tissues using TaqMan Universal Primer Mix, TaqMan Gene Expression Assays (FAM probe), with GAPDH as the housekeeping gene (VIC probe) in 384-well plates on a ThermoFisher Quantstudio 6 PCR machine.
5. ΔCT was calculated for the genes of interest relative to the housekeeping gene and expressed as $2^{-\Delta CT}$. Data analyzed via 2-Way ANOVA with Sex and Treatment as factors.

RESULTS

Organ	Gene of Interest	N (per group)	Average Expression Male NS	Male S	Female NS	Female S	P-Value
Hypothalamus	Corticotropin-Releasing Hormone	7	4.85E-5	1.24E-4	7.70E-5	4.08E-4	0.35
	Arginine Vasopressin	8	Expression below detectable threshold				
	Glucocorticoid Receptor	8	2.57E-2	2.61E-2	2.38E-2	2.23E-2	0.07 (Sex)
	Pro-opiomelanocortin	8	1.33E-3	1.34E-3	1.46E-3	3.02E-2	0.488
	Brain-Derived Neurotrophic Factor	8	Expression below detectable threshold				
Pituitary	CRH Receptor	8	1.49E-2	1.56E-2	1.78E-2	1.13E-2	0.11
	Vasopressin Receptor 1B	8	6.27E-5	8.07E-5	9.05E-5	8.07E-5	0.91
Adrenal Glands	Tyrosine Hydroxylase	5	1.57E-1	2.48E-1	1.57E-1	2.46E-1	0.22

CONCLUSION

1. Of the genes studied, Brain-Derived Neurotrophic Factor and Arginine Vasopressin in the Hypothalamus did not show consistent ΔCT values relative to the GAPDH housekeeping gene, and no there are no conclusive data to determine their role in stress-axis response.
2. No significant differences in gene expression were noted in CRH (Hypothalamus), POMC (Hypothalamus), Vasopressin Receptor 1B (Pituitary), and Tyrosine Hydroxylase (Adrenal Glands). These genes did not show a lasting differential level of expression in adult prenatally stressed rats.
3. A trend ($p = 0.07$) was noted in the levels of Glucocorticoid Receptor (GR) expression between sexes with no significant treatment effect. Average GR expression was higher in male rats than in female rats, suggesting that a basal difference in sensitivity to glucocorticoids may be present in adult animals independent of stress exposure. However, further study is needed to confirm this trend.
4. A trend ($p = 0.11$) was noted in CRH Receptor (CRHR1) expression. A Tukey-Kramer post-hoc analysis revealed a significant effect between both sex and treatment ($p = 0.04$), suggesting that sex-specific response to stress may become present with a larger sample size. However, the non-significant main effect requires further experimentation to confirm this trend.

STUDY IMPROVEMENTS

1. Failure in two of the eight genes relative to the housekeeping gene suggests alternate primers may be needed to show consistent expression. Alternatively, specific regions of the organs studied may show differential expression in genes and may require regional dissection prior to analysis.
2. The non-significant expression levels in the other genes studied may be the result of whole-organ homogenization. Analysis of expression in specific regions (such as the anterior lobe only in the case of corticotropin-releasing hormone) may offer greater specificity in terms of localized expression during stress response.

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